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(71) Applicant *(for all designated States except US)*: TANOX BIOSYSTEMS, INC. [US/US]; 10301 Stella Link Road, Houston, TX 77025–5497 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHEN, Alex [US/US]; LaJolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, CA 92121 (US). CHANG, Tse, Wen [US/–]; College of Life Sciences, National Tsing Hua University, Hsin–Chu (TW).

(74) Agent: MIRABEL, Eric, P.; Tanox Biosystems, Inc., 10301 Stella Link Road, Houston, TX 77025–5497 (US).

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(54) Title: INHIBITION OF ANTIGEN–SPECIFIC IgE PRODUCTION BY ANTIGEN COUPLED TO MEMBRANE IgE PETIDE

(57) Abstract

Disclosed is the use of the extracellular portion of the membrane–bound domain of the ∈ chain (from IgE) designated *migis–ϵ* peptides, or fragments or derivatives thereof, conjugated with antigen(s), for use in desensitization to such conjugated antigen(s). Two different isoforms of the *migis–ϵ* peptide are disclosed. These conjugates are administered to suppress IgE specific for the antigen of the conjugate, and therefore, suppress the allergic response to that antigen.

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Inhibition of Antigen-Specific IgE Production by Antigen Coupled to Membrane IgE Peptide

Field of the Invention

The invention relates to use of peptide-antigen conjugates to suppress IgE production in an antigen-specific manner, to desensitize a subject to the antigen of the conjugate.

Background of the Invention

Immunoglobulins consist of two peptide chains, a heavy chain and a light chain. There are five classes of immunoglobulins: IgG, IgM, IgA, IgD, and IgE. In IgE, the heavy chain is designated as the ϵ chain.

There are two forms of immunoglobulins: the secreted and the membrane-bound form. The membrane-bound form differs from the secreted form in that the former has a membrane-anchoring peptide extending from the C terminus of the ϵ chain. This membrane-anchoring peptide affixes the membrane-bound immunoglobulin to the cell membrane surface.

Membrane-anchoring peptides can be divided into three segments in terms of locations in relation to the plasma membrane. The middle segments have hydrophobic and uncharged amino acid residues, suggesting that they are in the membrane lipid bilayer. The C-terminal hydrophilic segments and have fewer amino acid residues, suggesting that they are intracellular. The segments toward the N-termini are highly acidic and hydrophilic, suggesting that they are on the extracellular surface of the plasma membrane.

The extracellular segments of these peptides are unique for different isotypes. Therefore, the extracellular segment of the ϵ chain membrane anchoring peptide forms, in whole or in part, an epitope unique to the B cells which produce IgE. However, this membrane-bound immunoglobulin isotype specific ("*migis*") extracellular epitope is not present on secreted, soluble IgE because only the immunoglobulin which is bound to the surface of B cells contains the membrane anchoring peptide as part of its heavy chain.

The immediate-type hypersensitivities, such as extrinsic asthma, hay fever, and allergic responses to certain foods or drugs, are mediated primarily by IgE. In an IgE-mediated allergic response, the allergen binds to the IgE which is bound to receptors on the surface of mast cells and basophilic leukocytes (basophils). The binding of the allergen causes crosslinking of the surface IgE molecules and hence the underlying receptors for the Fc portion of IgE (FcϵR), thereby triggering the release of pharmacologic mediators such as histamine, the slow-reacting substance of anaphylaxis (SRA), and serotonin. The release of these mast cell and basophil products causes the pathological reactions and symptoms of allergy.

IgE is secreted by a particular class of B cells, which also express IgE on their surface. In individuals sensitized to specific allergens, the allergen-specific IgE is continuously produced by these B cells. Nevertheless, individuals who have no secreted IgE in their systems (and no IgE-producing B cells) appear to

live normally, indicating that IgE is not essential in the immune response. IgE may, however, be useful in fighting infection by parasites.

It seems, therefore, that suppressing or depleting IgE would be a viable therapy for allergic diseases. Depleting IgE which binds to particular antigens would prevent those antigens from reacting to cause an allergic reaction.

Administration of antigens to reduce an allergic reaction on subsequent exposure to the antigens is known as desensitization. It is a widely accepted method of therapy for allergic diseases.

Summary of the Invention

The invention includes *migis*-ϵ peptides, or fragments or derivatives thereof, conjugated with antigens, or fragments or derivatives thereof. In the invention, these conjugates are administered to suppress IgE specific for the antigen of the conjugate, and therefore, suppress the allergic response to that antigen. Treatment with these conjugates will not result in IgE-anti-IgE complexes because the *migis*-ϵ sequence is absent in the secretory IgE, and antibodies generated against the *migis*-ϵ sequence, therefore, will not bind to the secretory IgE.

The invention also includes a number of variations and derivatives. There are two different isoforms of IgE present in humans, and either, or fragments or derivatives of either, can be conjugated to antigens and administered to reduce the IgE against that antigen. To reduce the antigen-specific IgE in mammals other than humans, one would use the *migis*-ϵ sequence from such mammal, conjugated

with an antigen of interest. This could be an effective veterinary treatment for allergic reactions caused by certain allergens such as flea allergy dermatitis in dogs, which results from flea bites.

Description of Making and Using the Invention

Immunization of mice with conjugates of an antigen and the mouse *migis*-ϵ peptide induced IgE-nonresponsiveness to that antigen on subsequent challenge with it. Rational extrapolation provides that immunization of humans or other mammals with a corresponding *migis*-ϵ peptide/antigen conjute would induce IgE-nonresponsiveness on subsequent challenge with that antigen. This would allow induction of IgE-nonresponsiveness to common allergens such as ragweed pollen, dust mite feces, cat and dog dander and saliva, or other common allergens. This would provide an effective method of allergen-specific desensitization.

For humans, two different isoforms of the *migis*-ϵ segment are known. The first is represented by amino acid numbers 4 to 18 of SEQ ID NO.:1 (Glu Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu Ala Pro Trp), and the second has this amino acid sequence 4 to 18 of SEQ ID NO.:1 spliced to the C terminal end of amino acid numbers 4 to 55 of SEQ ID NO.:2 (Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu Pro Arg Ala Ala Gly Gly Ser Val Pro His Pro Arg Cys His Cys Gly Ala Gly Arg Ala Asp Trp Pro Gly Pro Pro). Fragments, variant sequences, or derivatives, of either of these segments could also be used in the conjugates of the

invention. These segments could also be extended with additional amino acids or other moieties and used in the conjugates of the invention.

It is also possible to express conjugates including either isoform (or fragments or derivatives thereof) as fusion proteins, including the allergen(s) of interest. This would be a desirable production method for most peptide allergens. The invention also includes the nucleotide sequences for such fusion proteins, *i.e.*, an isoform with an allergen, as well as vectors and host cells including such nucleotide sequences.

The conjugates of the invention are preferably administered intravenously, subcutaneously, or intramuscularly, with an appropriate adjuvant. The dosages and administration regimen can be readily extrapolated from the animal data presented below.

Because of alternative mRNA splicings, there are two different nucleotide sequences which encode for peptides in the membrane anchoring region of human ϵ chain. The deduced amino acid sequences encoded by these two nucleotide sequences are also different, indicating that there are two different isoforms of the human ϵ chain membrane anchoring peptide.

The deduced amino acid sequence of isoform I shows that it has 67 amino acid residues, and a 15 amino acid peptide segment toward the N-terminus (SEQ ID NO:1). This 15 amino acid segment is proposed to be extracellular and to form, entirely or in-part, the *migis*-ϵ peptide. Isoform II has 119 amino acid residues, 67 of which are towards the N terminus and form the proposed

extracellular *migis*-ϵ segment (SEQ ID NO:2). Either isoform, or fragments or derivatives thereof, is appropriate for coupling to an antigen for use in the treatment method of the invention.

Example - Animal Model

Studies in mice have shown that a conjugate with an antigen and a *migis*-ϵ peptide can be a valuable therapeutic approach for desensitization to the antigen. These studies are described below.

Migis-ϵ peptide was selected from the mouse IgE genomic sequence, and had the sequence: Glu Leu Asp Ile Gln Asp Leu Cys Ile Glu Glu Val Glu Gly Glu Glu Leu Glu Glu Leu (SEQ ID NO.: 3). Secretory IgE peptides with some of the sequences from the CHϵ1 to CHϵ4 domains were also prepared. They had the sequences: Thr Thr Ser Gln Val Thr Ser Trp Gly Lys Ser Ala Lys Asn Phe Thr Cys His Val Thr (SEQ ID NO.: 4) (residue numbers 190-210 of CHϵ1); Gly Val Asp Tyr Leu Ala His Thr Arg (SEQ ID NO.: 5) (residue numbers 316-324 of CHϵ2); Pro Leu Asp Leu Tyr Gln Asn Gly Ala Cys (SEQ ID NO.: 6) (residue numbers 343-351 of CHϵ3). IgE peptides at 5 mg/ml were mixed with insulin B chain, BSA, KLH respectively, at 2 mg/ml in equal volumes to which glutaraldehyde was added at a final 0.05%, incubated at 25°C for 4 hr, and dialyzed. Monoclonal rat anti-mouse IgE antibodies EM 95 and BF815 were employed for the total IgE assay. Biotinylated rat anti-mouse kappa was obtained from Zymed (San Francisco, CA).

Eight week old female BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were grouped and were treated with *migis*-ϵ coupled to protein carriers. Sera were collected on day seven after the last immunization. Antigen-specific IgE was assessed by the passive cutaneous anaphylactic (PCA) skin test.

An anti-*migis*-ϵ assay was performed as follows. 50 μl *migis*-ϵ-BSA at 10 μg/ml were coated onto 96-well plate at 37°C for 1 hour. The plates were washed, blocked with Blotto, and added with 50 μl serum samples at appropriate dilutions. The plates were washed, incubated with biotinylated goat anti-mouse IgG or IgG subclasses, at 1 μg/ml for 1 hour at room temperature, washed, added with SA-AP, substrate, and read at 414 nM.

A total IgE sandwich assay was performed as follows. 96-well plates were coated with 50 μl MAb anti-ϵ, EM95, at 10 μg/ml overnight at 4°C, washed, blocked, added with sera at appropriate dilutions, biotinylated MAb anti-ϵ, BF815 was added, and plates developed as above.

An anti-IgE assay was performed as follows. Anti-NP IgE (lambda, ϵ) was used to coat the 96-well plates at 10 μg/ml overnight at 4°C. The plates were washed and blocked. Sera were added at appropriate dilutions, washed, followed by biotinylated rat anti-mouse kappa light chain, and developed as above.

migis-ϵ protein administered in complete and incomplete Freund's adjuvant (CFA/ICFA) inhibited IgE responses to the carrier protein. Adult BALB/c mice

were immunized five times i.p. with *migis*-ϵ-KLH (keyhole limpet hemocyanin) conjugates in CFA/ICFA, or in alum. Anti-KLH IgE responses were assessed in individual mice. A normal magnitude of anti-KLH IgE responses was observed in mice immunized i.p. with 10 μg KLH in CFA/ICFA, or in alum. In contrast, mice treated with 1 μg or 10 μg *migis*-ϵ-KLH in CFA/ICFA exhibited profoundly suppressed KLH specific IgE responses.

Migis-ϵ conjugated antigen did not affect antigen-specific IgG responses to the carrier. Comparable anti-KLH IgG1 responses were observed in KLH or *migis*-ϵ-KLH immunized mice, while alum favored antigen-specific IgG1 production over CFA/ICFA. Higher levels of anti-*migis*-ϵ of IgG1 subclass were observed in mice immunized with *migis*-ϵ-KLH in alum. In contrast, anti-KLH and anti-*migis*-ϵ of IgG2a and IgG2b subclasses were present in higher concentrations in mice immunized with *migis*-ϵ-KLH in CFA/ICFA. However, suppression of anti-KLH IgE responses appeared not directly correlated with the levels of different subclasses of anti-*migis*-ϵ antibodies. Although anti-KLH IgE was suppressed in *migis*-ϵ-KLH treated mice, total IgE levels appeared to be normal in mice immunized with *migis*-ϵ-KLH emulsified in CFA/ICFA.

To ascertain that suppression of KLH responses was not due to alteration of protein carriers by chemical coupling, synthetic peptides corresponding to the CHϵ1 to CHϵ4 domains, as well as insulin B chain (InB), were coupled to KLH by glutaraldehyde under similar conditions. Comparable magnitude of anti-KLH IgE responses was observed in mice immunized with KLH coupled to SEQ ID

NOS:4 to 6 or Insulin B chain in CFA/ICFA, whereas anti-KLH IgE responses were suppressed in mice treated with *migis*-ϵ-KLH.

Suppression of IgE responses to *migis*-ϵ conjugated proteins did not affect a concomitant unrelated antigenic challenge. To examine whether *migis*-ϵ-KLH treatment may suppress IgE responses to an unrelated antigen, mice were pretreated with 1 to 50 μg *migis*-ϵ-KLH in CFA/ICFA, or with 10 μg *migis*-ϵ-KLH in CFA twice, followed by a challenge with *migis*-ϵ-KLH along with OVA in ICFA, and further boosted with OVA/*migis*-ϵ-KLH in ICFA twice. KLH administered in CFA/ICFA, inhibited IgE responses against the KLH to which *migis*-ϵ was coupled, but did not inhibit IgE responses against an unrelated OVA antigenic challenge. In contrast, mice treated with glutaraldehyde-modified KLH from 1 to 50 μg in CFA/ICFA exhibited normal levels of anti-KLH, and anti-OVA IgE responses.

To test whether suppression of *migis*-ϵ-KLH may be extended to other *migis*-ϵ conjugated antigens, BALB/c mice were injected with 20 μg soluble *migis*-ϵ-BGG or glutaraldehyde modified BGG (GA-BGG) subcutaneously, or intraperitoneally. Mice were then challenged with *migis*-ϵ-BGG plus OVA, or BGG plus OVA in alum. Mice treated with soluble *migis*-ϵ-BGG via either route, failed to elicit anti-BGG IgE when challenged with *migis*-ϵ-BGG or BGG in alum, whereas anti-OVA IgE responses in these mice were normal. As a control, treatment with GA-BGG via either route did not affect subsequent anti-BGG or anti-OVA IgE responses.

Moreover, *migis*-ϵ conjugated BGG did not affect anti-BGG IgG responses. Comparable anti-BGG or anti-OVA IgG responses were observed in mice treated with *migis*-ϵ-BGG and GA-BGG. Anti-*migis*-ϵ IgG was not detectable in mice treated with soluble *migis*-ϵ-BGG. *migis*-ϵ-BGG treatment did not augment the production of anti-IgE nor modulate the levels of total IgE levels. Moreover, total IgE as well as basal levels of anti-IgE antibodies were also comparable in mice treated with *migis*-ϵ-BGG or BGG as control.

Thus, it can be seen that inhibition of antigen-specific IgE production can be achieved by treatment with *migis*-ϵ conjugated antigens. The following were observed: a) Inhibition of anti-KLH and anti-BGG IgE, but not IgG responses was observed in mice treated with soluble or *migis*-ϵ-conjugated protein emulsified in CFA/ICFA. b) Inhibition was observed in IgE responses to *migis*-ϵ conjugated carrier protein, but not toward an unrelated antigen. c) Inhibition of antigen-specific IgE was not correlated with levels of anti-*migis*-ϵ or anti-IgE antibodies; d) total IgE levels remained comparable among mice treated with *migis*-ϵ conjugated antigens and native or glutaraldehyde-modified carrier antigen as control.

If conjugates were designed for use in humans, with one of the isoforms or a fragment or derivative thereof, as shown in SEQ ID NOS.: 1 and 2, conjugated with an antigen, the same results would be expected. That is, one would expect to see: a) inhibition of antigen-specific IgE, but not IgG responses; b) no inhibition of IgE responses to unrelated, unconjugated antigens; c) no

correlation between inhibition of antigen-specific IgE and levels of anti-*migis*-ϵ or anti-IgE antibodies; d) total IgE levels would remain comparable among subjects treated with *migis*-ϵ conjugated antigens and those exposed to the native antigen. This would be an effective method of desensitizing human subjects to allergens.

The terms, expressions and examples herein are exemplary only and not limiting, and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. All such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) General Information:
(i) Applicant: Chen, Swey-Shen Alex; Chang, Tse Wen
(ii) Title of Invention: Inhibition of Antigen-Specific IgE Production by Antigen Coupled to Membrane IgE Peptide
(iii) Number of Sequences: 6
(iv) Correspondence Address:
(A) Addressee: Tanox Biosystems, Inc.
(B) Street: 10301 Stella Link Rd.
(C) City: Houston
(D) State: Texas
(E) Country: USA
(F) Zip: 77025
(v) Computer Readable Form:
(A) Medium Type: Diskette, 3.5 inch
(B) Computer: IBM PS/2
(C) Operating System: DOS 3.30
(D) Software: Wordperfect 5.1
(vi) Current application data:
(A) Application Number:
(B) Filing Date:
(C) Classification:
(vii) Prior Application Data:
(A) Application Number:
(B) Filing Date:
(viii) Attorney/Agent Information:
(A) Name: Mirabel, Eric P.
(B) Registration Number: 31,211
(C) Reference/Docket Number: TNX97-2-PCT
(ix) Telecommunication Information:
(A) Telephone: (713) 664-2288
(B) Telefax: (713) 664-8914
(2) Information for SEQ ID NO:1:
(i) Sequence Characteristics:
(A) Length: 216 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:1:

```
GTA AAT CCC GAG CTG GAC GTG TGC GTG  27
Val Asn Pro Glu Leu Asp Val Cys Val
1               5

GAG GAG GCC GAG GGC GAG GCG CCG TGG ACG 57
Glu Glu Ala Glu Gly Glu Ala Pro Trp Thr
10                  15

TGG ACC GGC CTC TGC ATC TTC GCC GCA CTC 87
Trp Thr Gly Leu Cys Ile Phe Ala Ala Leu
20                  25

TTC CTG CTC AGC GTG AGC TAC AGC GCC GCC 127
Phe Leu Leu Ser Val Ser Tyr Ser Ala Ala
30                  35

CTC ACG CTC CTC ATG GTG CAG CGG TTC CTC 157
Leu Thr Leu Leu Met Val Gln Arg Phe Leu
40                  45

TCA GCC ACG CGG CAG GGG AGG CCC CAG ACC  187
Ser Ala Thr Arg Gln Gly Arg Pro Gln Thr
50                  55

TCC CTC GAC TAC ACC AAC GTC CTC CAG CCC  207
Ser Leu Asp Tyr Thr Asn Val Leu Gln Pro
60                  65

CAC GCC TAG 216
His Ala
70
```

(2) Information for SEQ ID NO:2:

(i) Sequence Characteristics:

(A) Length: 166 nucleotides

(B) Type: nucleic acid

(C) Strandedness: double stranded

(D) Topology: linear

(xi) Sequence Description: SEQ ID NO:2:

```
GTA AAT CCC GGG CTG GCT GGC GGC TCC GCG 30
Val Asn Pro Gly Leu Ala Gly Gly Ser Ala
1               5                   10

CAG TCC CAG AGG GCC CCG GAT AGG GTG CTC 60
Gln Ser Gln Arg Ala Pro Asp Arg Val Leu
                15                  20
```

```
TGC CAC TCC GGA CAG CAG CAG GGA CTG CCG 90
Cys His Ser Gly Gln Gln Gln Gly Leu Pro
                25                  30

AGA GCA GCA GGA GGC TCT GTC CCC CAC CCC 120
Arg Ala Ala Gly Gly Ser Val Pro His Pro
                35                  40

CGC TGC CAC TGT GGA GCC GGG AGG GCT GAC 150
Arg Cys His Cys Gly Ala Gly Arg Ala Asp
                45                  50

TGG CCA GGT CCC CCA G 166
Trp Pro Gly Pro Pro
                55
```

(2) Information for SEQ ID NO:3:
(i) Sequence Characteristics:
(A) Length: 20
(B) Type: amino acid
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:3:

```
Glu Leu Asp Ile Gln Asp Leu Cys Ile Glu Glu Val
1               5                   10

Glu Gly Glu Glu Leu Glu Glu Leu
        15                  20
```

(2) Information for SEQ ID NO:4:
(i) Sequence Characteristics:
(A) Length: 20
(B) Type: amino acid
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:4:

```
Thr Thr Ser Gln Val Thr Ser Trp Gly Lys Ser Ala Lys
1               5                   10

Asn Phe Thr Cys His Val Thr
    15                  20
```

(2) Information for SEQ ID NO:5:
(i) Sequence Characteristics:
(A) Length: 9
(B) Type: amino acid
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:5:

Gly Val Asp Tyr Leu Ala His Thr Arg
1 5

(2) Information for SEQ ID NO:6:
(i) Sequence Characteristics:
(A) Length: 10
(B) Type: amino acid
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:6:

Pro Leu Asp Leu Tyr Gln Asn Gly Ala Cys
1 5 10

What Is Claimed Is:

1. A conjugate comprising an antigenic molecule coupled to a peptide, said peptide including all of or a fragment or derivative of the *migis*-ϵ peptide.

2. The conjugate of claim 1 wherein said *migis*-ϵ peptide has the sequence of amino acid numbers 4 to 18 of SEQ ID NO:1.

3. The conjugate of claim 1 wherein said *migis*-ϵ peptide has the sequence of amino acid numbers 4 to 55 of SEQ ID NO:2 with amino acid numbers 4 to 18 of SEQ ID NO:1 attached to its C terminal end, or the *migis*-ϵ peptide is a fragment of such peptide.

4. A method of desensitization to an antigenic molecule comprising immunizing with the conjugate of any of claims 1 to 3.

5. A method of reducing the amount of antigen-specific IgE comprising administering a conjugate comprising an antigen coupled to a peptide, said peptide including all of or a fragment or derivative of the *migis*-ϵ peptide.

6. The method of claim 5 wherein said peptide has the sequence of amino acid numbers 4 to 18 of SEQ ID NO:1.

7. The method of claim 5 wherein said peptide has the sequence of amino acid numbers 4 to 55 of SEQ ID NO:2 with amino acid numbers 4 to 18 of SEQ ID NO:1 attached to its C terminal end, or a fragment of such peptide.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11707

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/04, 38/10; C07K 7/08, 14/00, 4/12
US CL :530/324, 325, 326, 327, 328, 329, 387.1, 405

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326, 327, 328, 329, 387.1, 405

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,254,671 A (CHANG) 19 October 1993, column 10, lines 3-9.	1
X	US 5,274,075 A (CHANG) 28 December 1993, columns 8-10.	1 and 5
X	US 5,281,699 A (CHANG) 25 January 1994, column 16, lines 16-23.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
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"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 SEPTEMBER 1997	Date of mailing of the international search report 06 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LAURIE SCHEINER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11707

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2-4, 6 and 7
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

the claims are limited to specific sequence identifiers, however, a sequence disk was not submitted. In the absence of the sequences in computer readable form, claims 2-4, 6 and 7 are unsearchable.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.